The effects of antioxidants in the senescent auditory cortex

C. de Rivera [a], B. Shukitt-Hale [b], J.A. Joseph [b], J.R. Mendelson [a],*

[a] *Department of Speech-Language Pathology, University of Toronto, Toronto, Ont., Canada M5G 1V7*
[b] *United States Department of Agriculture Human Nutrition Research Center on Aging at Tufts, Boston, MA 02111, USA*

Abstract

We investigated whether a 2-month dietary supplementation of antioxidants, in the form of blueberry phytochemicals, could reverse or retard the age-related decline in temporal processing speed observed in the aged rat. To this end, extracellular single unit responses to frequency modulated (FM) sweeps were recorded in the primary auditory cortex (AI) of aged rats that had been placed on either a blueberry-supplemented or control diet 2 months prior to the physiological recordings. Results showed that most cells recorded from the blueberry-fed rats responded most vigorously to fast FM sweeps, similar to that observed in young rats. In contrast, the majority of cells recorded from the control rats showed a preference for slow FM sweep rates. These results suggest that age-related changes in temporal processing speed in A1 may be reversed by dietary supplementation of blueberry phytochemicals.
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1. Introduction

Age-related hearing loss is among the most common problems affecting the elderly population. One of its more debilitating forms is a difficulty in discriminating speech, even in a quiet environment [3,40]. One possible cause may be an age-related decline in processing speed throughout the central nervous system (CNS) [42,56]. For the senescent auditory system, a deterioration in processing the rapidly changing consonant–vowel formant transitions (which are comprised of rapid changes in frequency and amplitude over time), may be an important source of diminished speech discrimination in the elderly [21,32,45,56]. Unfortunately, relatively few studies have explored the effects of aging on processing these more dynamic aspects of speech [45,49,54].

One stimulus that lends itself well to investigate the effects of aging on temporal processing speed in the auditory system is the frequency modulated (FM) sweep. FM sweeps, which are characterized by changes in frequency over time, share features in common with formant transitions. Recently, we found that cells in the primary auditory cortex (AI) of aged rats respond more vigorously to slower as compared to faster FM sweeps [32]. These results suggest the presence of an age-related decline in the rate of change of frequency that can be processed by the auditory cortex.

In recent years a great deal of research has been devoted to characterizing age-related changes in the CNS in an effort to develop techniques that can halt or even reverse these changes. One such area has focused on attempting to reduce the amount of oxidative stress (OS) that accompanies aging [2,37,57]. Researchers have found that by increasing the amount of antioxidants in the organism's system some of the debilitating effects of aging can be reversed. For example, we have showed that an antioxidant-enriched diet impedes the onset of age-related neuronal signal transduction and cognitive behavioural deficits in rats [18]. We also found that aged rats placed on a diet supplemented with blueberries for 2 months showed less of an age-related decline than control animals [16]. In fact, these animals showed an improvement in motor and cognitive behaviours when compared to control animals. In addition, in an in vitro model, antioxidants

* Corresponding author. Present address: Toronto Rehabilitation Institute, University Centre, 550 University Ave., Toronto, Ont., Canada M5G 2A2. Tel.: +1 416 597 3422x3852.

E-mail address: mendelson.julie@torontorehab.on.ca (J.R. Mendelson).

from blueberries were shown to ameliorate the experimentally induced formation of reactive oxygen species (ROS) in red blood cells [58].

Recently, researchers have found a negative correlation between ROS and presbycusis [4,20,47]. Using an in vitro model, researchers were able to demonstrate that antioxidants from blueberries improved the experience-induced formation of ROS [58]. In the auditory system, studies have demonstrated that OS plays a deleterious role in noise exposure and ototoxicity [14,22].

While the effects of antioxidants appear to be encouraging little is known about their potential effects on sensory processing, particularly in the auditory system. Because ROS causes pathological changes in the morphology of neurons [8,24,50], such detrimental modifications could compromise normal cell functioning and potentially act to disrupt synaptic activity. This in turn could affect temporal processing speed within the auditory system. Thus, the goal of the present study was to determine if the introduction of antioxidants into the diet of aged rats would affect auditory cortical temporal processing speed. Specifically, we asked if an antioxidant-enriched diet containing blueberry phytochemicals would reverse the effects of aging on temporal processing speed of speech-related acoustic signals.

2. Materials and methods

2.1. Subjects

Twenty-six young (2–4 months old; ∼300–400 g) male Long Evans Hooded rats (Charles River Laboratories) were aged for 23 months at the University of Toronto Animal Care Facility. Rats were initially housed in pairs in standard laboratory cages and kept on a 12-h light/12-h dark cycle with ad libitum access to food and water. At 6 months of age, rats were housed individually and placed on a restricted diet of 7 normal rat chow pellets daily. This was done to ensure maintenance of their current weight and to avoid excessive weight gain, which can lead to premature death. Previous research from our laboratory has shown this to be a sufficient amount of food [23,32,34]. The ears were checked regularly with an otoscope and were shown to be clear of any debris.

2.2. Feeding protocol

At 23 months rats were randomly assigned to one of three diet groups. The experimental group (A-BB) was placed on an antioxidant-enriched diet and fed 12 specially formulated corn pellets supplemented with blueberry extract (for preparation procedure see [58]). The first control group (A-CR) continued to receive seven pellets of regular laboratory rat chow. The second control group (A-CC) received 12 corn pellets daily. The blueberry pellets consisted of the same ingredients as the corn pellets (see Table 1) with

the addition of just 2% blueberry extract [58]. The difference in the number of pellets given to the A-BB and A-CC groups compared to the A-CR (12 versus 7) was to offset the consistency of the different pellets and ensure that all rats received the same net amount of food needed to maintain their weight. Thus, although the A-CR group were given fewer pellets, it actually recieved the same net amount of food found in the 12 pellets served to the A-CC group. The inclusion of the second control group, A-CC, was to ensure that there was no effect from the corn component of the pellets.

2.3. Surgical procedure

The surgical procedures employed in this study have been described elsewhere and thus, will only be summarized here [32,39]. The procedures have been approved by the Canadian Council for Animal Care and comply with the stipulations regarding the care and use of experimental animals set out by the American Physiological Association. Briefly, rats were initially weighed and anaesthetized with Equithesin (3 mg/kg i.p.) followed by administration of 0.1 cm^3 subcutaneous injection of atropine sulfate to prevent respiratory distress. They were then placed in a modified stereotaxic apparatus that allowed for unobstructed access to the pinnae. A craniotomy was then performed over the auditory cortex. All wound margins and pressure points were generously infiltrated with a long-lived local anesthetic (bupivacaine hydrochloride 2.5%). The exposed brain was bathed with silicone oil (Dow Corning Co.) in order to prevent it from drying out and to reduce any possible pulsations. An areflexive level of anaesthesia was maintained by supplemental i.p. injections of Equithesin. Body temperature was maintained at 37.5 °C by a thermostatically controlled heating pad.

2.4. Stimulation and recording

All experiments were conducted in an electrically shielded, sound-attenuating chamber (IAC). Extracellular single unit responses were recorded using teflon-coated tungsten microelectrodes (impedance 0.7–1.2 MΩ at 1.0 kHz). Recording procedure and equipment have been previously described [32,39].

Stimuli were fed to a pair of calibrated STAX (SR 54) earphones encased in small chambers that were connected to sound delivery tubes sealed into the acoustic meatuses (Sokolich US Patent 4,251,686; 1981). The speculae of the couplers fit snugly into the external meatuses to within 2 mm of the tympanic membranes. Calibration of pure tone stimuli was carried out in situ with a B&K 1/4-in. microphone (4136) and corrected for on-line analysis.

Stimuli were generated and presented by a Macintosh computer and the MALab auditory system. Two types of tonal stimuli were employed: (1) pure tone bursts (100 ms duration with a 5 ms rise/decay time) and (2) linear FM sweeps iden-

Table 1
List of ingredients for both types of control diets and experimental diet

Control A (CR)	Control B (CC)	Experimental (BB)
Wheat germ meal	Wheat, hard ground	Wheat, hard ground
Wheat middlings	Wheat middlings	Wheat Middlings
Oast, ground	Oats, ground	Oats, ground
Fish meal	Fish meal, Menhaden, 60%	Fish meal, menhaden, 60%
Soybean meal	Soybean meal, 48%	Soybean meal, 48%
Alfalfa meal, dehydrated	Alfalfa meal, dehydrated, 17%	Alfalfa meal, dehydrated, 17%
Meat meal	Corn gluten meal, 60%	Corn gluten meal, 60%
Calcium phosphate	Dicalcium phosphate, 18.5%	Dicalcium phosphate, 18.5%
Animal fat with BHA	Soybean oil	Soybean oil
Brewer's yeast dried	Brewer's yeast dried	Brewer's yeast dried
Salt	Salt, ionized NaCl	Salt, ionized NaCl
Calcium carbonate	Calcium carbonate, 38%	Calcium carbonate, 38%
Vitamin mix[a]	Vitamin mix, NIH-31[c]	Vitamin mix, NIH-31[c]
Mineral mix[b]	Mineral mix, NIH-31[d]	Mineeral mix, NIH-31[d]
Corn, yellow ground	Corn, yellow ground	Corn, yellow ground
Dried beet pulp		Tif-blue blueberry extract
Cane molasses		
Spraydried whey		
Di-methionine		
Sodium selenite		

[a] Vitamin mix contains: choline chloride, calcium D-pantothenate, Vitamin B12, Di-alpha-tocopherol acetate, niacin, Vitamin A acetate, Vitamin A palmitate, riboflavin, D-biotin, folic acid, pyridoxine HCl, menadione sodium bisulfite, thiamine mononitrate.

[b] Mineral mix contains: ferrous carbonate, manganous oxide, copper sulphate, zinc oxide, zinc sulphate, calcium iodate, cobalt carbonate.

[c] Vitamin mix NIH-31 contains: corn ground, choline chloride, MSB complex, thiamin mononitrate, calcium panthothenate, Vitamin B12, Vitamin E, niacin, Vitamin A Acetate, riboflavin Biotin, Vitamin D3, folic acid, pyridoxine HCl.

[d] Mineral mix NIH-31 contains: magnesium oxide, calcium carbonate, ferrous sulfate, manganous oxide, copper sulfate, zinc oxide, calcium iodate, cobalt carbonate.

tical to those used in our previous research [23,25,31,32,39]. FM sweeps extending from 0.15 to 45.0 kHz (upward-directed) or from 45.0 to 0.15 kHz (downward-directed) were used. Rates of frequency change (i.e., sweep speeds) were 0.03, 0.05 (corresponding to slow speeds), 0.3 (corresponding to medium speeds) and 0.8 kHz/ms (corresponding to fast speeds). In previous studies from our laboratory these rates were empirically determined to be effective and representative in evoking unit responses [30,32,39]. While additional speed conditions could have been used, our previous studies showed that the 4 used here were typically the speeds to which most cells responded. It is interesting to note that the rates of frequency change contained in most rat vocalizations occur at slower speeds than those used here [19]. However, when rats are engaged in aggressive or sexual behaviours, rates of frequency change occur at faster speeds comparable to those used in the present study [19]. Frequency excursion and speed were kept constant within a trial while speed was varied from one set of trials to the next. Thus, the sweep durations for the different speed conditions were 56.2 ms for 0.8 kHz/ms, 150 ms for 0.3 kHz/ms, 900 ms for 0.05 kHz/ms and 1500 ms for 0.03 kHz/ms. Each FM sweep was preceded by a 200 ms interval containing the upper or lower starting frequency of the sweep in order to avoid the risk of contaminating the data with any equipment-related broadband transient click at the beginning or end of each FM sweep presentation. For all cells, the initial and end frequencies were always well outside the cell's response area. FM sweeps were presented at a rate of 1 sweep every 3 s.

2.5. Procedure

Extracellular single units were recorded along penetrations aimed oblique to the surface of AI. The majority of recordings for both age groups were made between layers III and V. Either at the beginning or at the end of an experiment the frequency representation of the cortical surface in the immediate vicinity was mapped to establish the relative location of recording sites within AI. In all cases, the frequency maps thus obtained were consistent with previous descriptions of the frequency representation in AI [41].

Once an auditory response was obtained, the unit's characteristic frequency (CF) and threshold were determined for the contralateral ear using pure tone stimuli. Following this, FM sweeps (two directions at four speeds) were presented monaurally to the contralateral ear at 25–30 dB above threshold (as determined in response to the CF pure tone stimulus). FM sweeps were presented at this intensity in order to (1) attempt to offset any elevated thresholds and to (2) effectively stimulate the greatest number of both monotonic and non-monotonic units [33,46]. Each sweep condition was presented 10 times. In previous studies from our laboratory, we presented each stimulus 40 times. However, when we compared the responses of the first 10 presentations with the subsequent 30 presentations, we found no difference in the preferred speed or direction of the FM sweep. Thus, in order to maximize the number of units that we could record from an individual animal, we reduced the number of presentations to 10.

Neurons recorded from the same penetration had to be at least 200–300 μm apart in order to ensure that the recordings were not made from the same cell. In addition, the distance between each penetration was at least 300 μm. This yielded, on average, 2–3 units per electrode penetration with approximately 2–3 penetrations per animal. The penetrations were randomly made within the cortex and were spaced such that a wide range of frequencies were sampled within a given animal. In this way, we endeavoured to reduce the risk of biasing our sample especially since a topographical distribution of FM sweep direction has been found in rat AI [60]. There was no individual animal where recordings were made from a small frequency region. At the end of each experiment, all animals were sacrificed with an overdose of somnotol.

2.6. Data analysis

Spike activity was recorded for the entire 3 s trial and was collected in bins of 5 ms duration. The peak firing rate (PFR in spikes/s) of neuronal responses to FM sweeps was quantified for all cells. PFR was determined by noting the bin containing the greatest number of spikes. For studies using FM sweeps where speed is a variable, PFR is preferable to the total number of spikes due to the fact that FM sweeps of different speeds spend different amounts of time within the excitatory response area for a given neuron. Thus, an analysis based on total spike counts would introduce an artifactual bias in favour of slower speeds. PFR is a well-established method for analyzing these types of data [30,32,35,39].

Preferred speed was determined by noting the rate of frequency change that elicited the largest PFR. Preferred direction of FM sweep was assessed by comparing responses to upward- and downward-directed FM sweeps at the preferred speed. An index of direction selectivity was calculated using the following equation:

$$DS = \frac{RU - RD}{RU + RD}$$

where DS is the index of direction selectivity and RU and RD are the responses (expressed as PFR in spikes/s) to upward- and downward-directed FM sweeps, respectively. This measure of direction selectivity has the advantage of being relatively insensitive to absolute firing rate. Cells were considered direction-selective if their absolute direction selectivity index value was equal to or greater than 0.33, which corresponds to a response to one direction that is twice as large as the response to the opposite direction. This criterion value of direction selectivity has been used in studies of the visual [11] as well as the auditory system (e.g., [10,30]). We used the sign of the direction selectivity index value to classify the direction of the selective response. Thus, a direction selectivity index value equal to or greater than +0.33 corresponded to a preference for upward-directed FM sweeps, while a direction selectivity

index value equal to or less than −0.33 was associated with a selectivity for downward-directed FM sweeps.

3. Results

A total of 249 cells were studied from the auditory cortex of 26 aged rats. Eighty-seven units were recorded from 9 rats in the A-BB group, 88 units from 10 rats in the A-CC group and 74 units from 14 rats in the A-CR group. This latter group included 38 units (from 7 rats) that had previously been reported in another study from our laboratory [32]. We compared the data from the A-CR group with these previously published data [32] and found no significant differences for any of the parameters examined, including preferred speed (χ^2:3; d.f. = 4.6; $p > 0.05$) and direction (χ^2: 2; d.f. = 0.8; $p > 0.05$) of FM sweep [32]. Thus, we combined the data from these two groups into a single group. All units showed speed and/or direction selectivity for the FM sweep.

While the number of units recorded from individual animals was relatively small, there appeared to be little variation from animal to animal within a group. Thus, for example, the majority of units recorded from each rat in the BB-fed group preferred fast FM sweeps. The same was true for the other groups; namely that each animal within a group displayed the characteristic response properties for that group.

3.1. Comparisons between the three diet groups

Fig. 1A shows an example of the type of response recorded from a rat maintained on the blueberry-enriched diet. This cell preferred the fast and medium speeds of FM sweep. In addition, it preferred upward-directed FM sweeps. The vast majority (90.6%) of the A-BB units preferred fast or medium speeds of FM sweeps while relatively few preferred the slower sweeps (Fig. 1B). For direction selectivity, the majority of units (59.3%) were non-direction-selective (Fig. 1C). However, of the direction-selective cells the majority (82.8%) preferred upward-directed FM sweeps.

The response of a cell recorded from a rat in the A-CC group is illustrated in Fig. 2A. This cell preferred the slow FM sweep speed of 0.05 kHz/ms. Additionally, this cell was direction-selective for upward-directed sweeps. Unlike rats maintained on the blueberry-enriched diet, the majority of units recorded from corn-fed rats (65.4%) preferred slower speeds of FM sweeps (Fig. 2B). For preferred direction, the majority of units (58.3%) were non-direction-selective (Fig. 2C). Further, as observed with cells from the BB group, the majority of direction-selective units (74.2%) preferred upward-directed sweeps.

An example of a unit recorded from the A-CR group is shown in Fig. 3A. As can be seen, this unit responded most vigorously to the slow and medium speeds. Further, it was direction-selective for upward-directed sweeps at the medium speed of 0.3 kHz/ms. As with the A-CC rats, the majority of units recorded from the A-CR group (67.4%) preferred slower FM sweep speeds (Fig. 3B). For preferred direc-
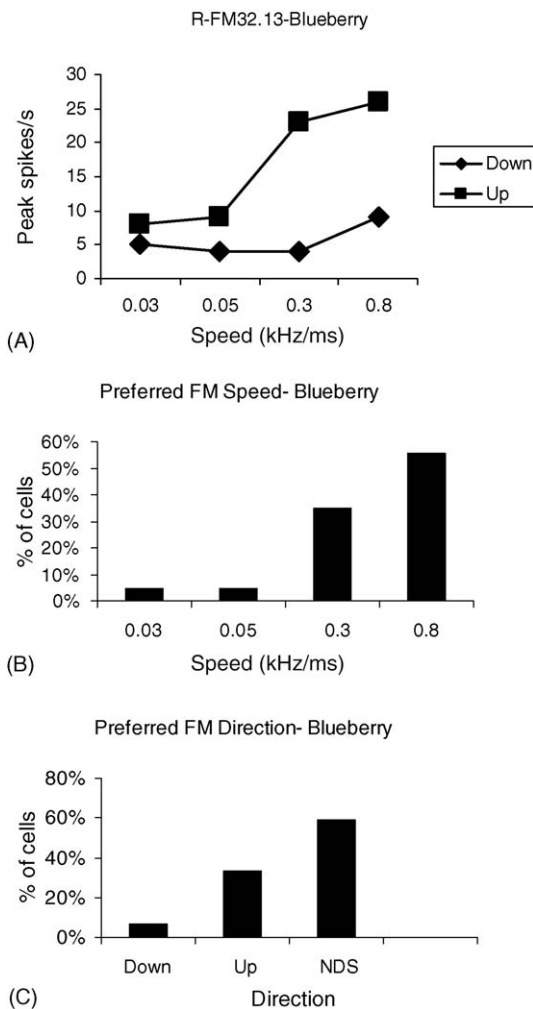
Fig. 1. Responses of cells recorded from animals maintained on the blueberry supplemented (A-BB) diet. (A) An example of a direction-selective cell that preferred upward-directed sweeps (squares) (B) For preferred speed the majority of cells responded best to the medium and fast speeds. (C) For preferred direction, over half of the cells were non-direction-selective. However, of the direction-selective cells, most preferred the upward-directed sweeps. Squares correspond to upward-directed FM sweeps while diamonds correspond to downward-directed FM sweep.
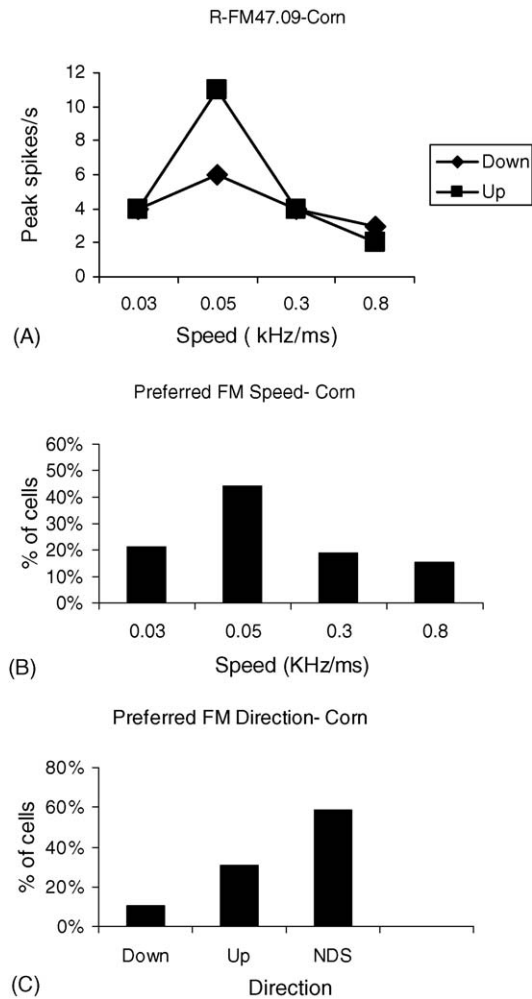
Fig. 2. Responses of cells recorded from animals maintained on the control corn supplemented (A-CC) diet. (A) An example of a direction-selective cell that preferred the slow speed of 0.05 kHz/ms. (B) For preferred speed the majority of cells responded best to the slower speeds. (C) For preferred direction, over half of the cells were non-direction-selective. Of the direction-selective cells, most preferred the upward-directed sweeps. Conventions are the same as in Fig. 1.

tion, just under half of the units (45.9%) were non-direction-selective (Fig. 3C). However, similar to the A-BB and A-CC groups, the majority of direction-selective units (72.5%) in the A-CR group preferred upward-directed sweeps.

Using the Holm Procedure to correct for multiple tests, comparisons were made between the three diet groups (Fig. 4A). There was no significant difference in speed preference between cells recorded from rats in the A-CC and A-CR groups (χ^2: 3; d.f. = 1.05; $p > 0.05$). However, there was a significant difference in speed preference between the A-BB and A-CC groups (χ^2: 3; d.f. = 59.8; $p < 0.0001$) and the A-BB and A-CR groups (χ^2: 3; d.f. = 59.9; $p < 0.0001$). This suggests that rats fed a diet supplemented with blueberry phytochemicals do not suffer from the same deleterious effects of aging on temporal processing speed as do rats on non-antioxidant-enriched diets.

Fig. 4B shows the distribution of direction-selective responses for the three diet groups. Statistical analysis yielded no significant differences in direction selectivity between the three diet groups (A-CC and A-CR: χ^2: 2; d.f. = 2.4; $p > 0.05$; A-BB and A-CC: χ^2: 2 d.f. = 0.78; $p > 0.05$; A-BB and A-CR: χ^2: 2; d.f. = 4.0; $p > 0.05$).

3.2. Comparisons between young and aged animals

We compared the data from the present study with data ($n = 55$) that we had previously collected from young rats maintained on a diet of regular laboratory rat chow (Y-CR) [39]. As described previously the majority of units (67%) recorded from the Y-CR rats preferred faster speeds. When we compared this to the data reported here we found it to be significantly different from aged rats in the A-CC group (χ^2 3; d.f. = 16.6; $p < 0.001$) and the A-CR group (χ^2 3; d.f. = 16.2;
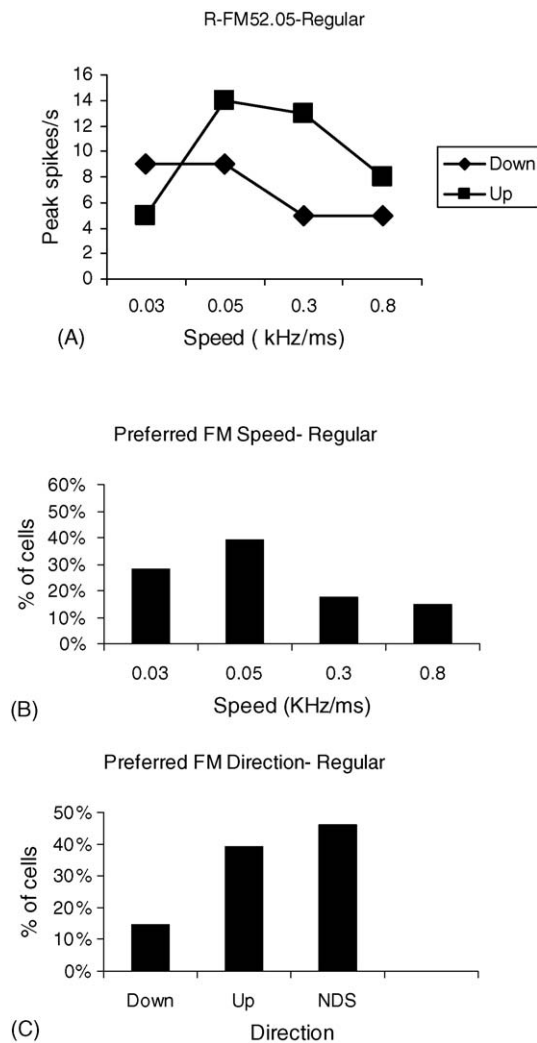
(A)

(B)

(C)

Fig. 3. Responses of cells recorded from animals maintained on regular laboratory (A-CR) chow. (A) This cell was direction-selective for upward-directed FM sweeps (squares) and preferred slow (0.05 kHz/ms) and medium (0.3 kHz/ms) speeds. For preferred speed the majority of cells responded best to the slower speeds. For preferred direction, just over half of the cells were direction-selective with the majority of these cells preferring the upward-directed sweeps. Conventions are the same as in Fig. 1.
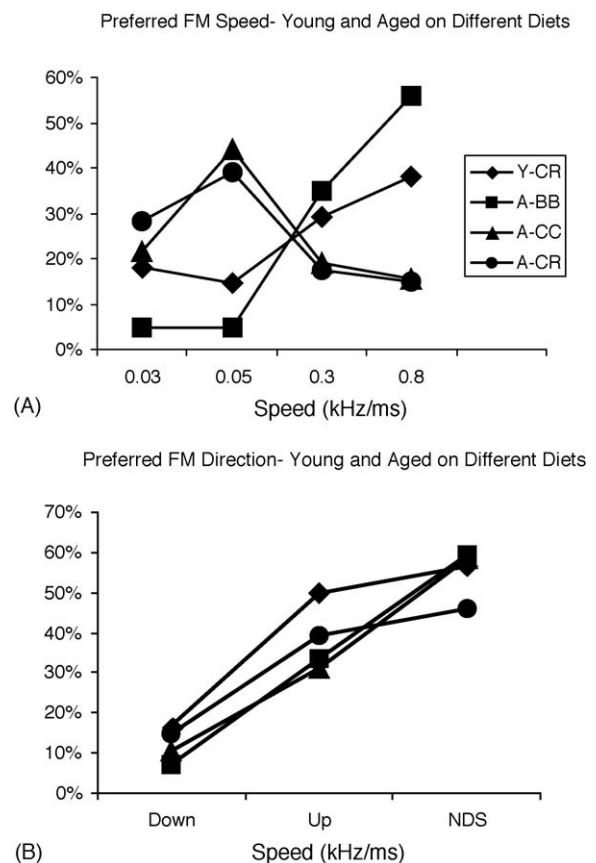

(A)

(B)

Fig. 4. Distribution of preferred speed (A) and preferred direction (B) responses for young and aged animals. (A) Cells recorded from rats in the A-BB (squares) and Y-CR (diamonds) groups preferred the faster sweeps. On the other hand, slow speeds were preferred by cells recorded from rats in the A-CC (triangles) and A-CR (circles) groups. (B) For preferred direction, the only group that had a majority of direction-selective units was the A-CR group. However, in both age groups and all diet types, of the direction-selective cells, the majority preferred upward-directed FM sweeps. Diamonds: Y-CR, squares: A-BB, triangles: A-CC, and circles: A-CR.

$p < 0.001$). Somewhat surprising is that it was also significantly different than what we observed in the A-BB group (χ^2 3; d.f. = 15.4; $p < 0.001$; Fig. 4A).

Fig. 4B shows the comparison of direction selectivity between the aged rats in the three diet groups and the young rats. Unlike what we observed with preferred speed, there were no significant differences in direction-selective responses between the young animals and the other three groups: Y-CR versus A-CR (χ^2 2; d.f. = 1.4; $p > 0.05$); Y-CR versus A-CC (χ^2 2; d.f. = 0.06; $p > 0.05$) and Y-CR versus A-BB (χ^2 2; d.f. = 0.67; $p > 0.05$). In addition, of the direction-selective cells, the majority of units in all four groups preferred upward-directed sweeps. These results suggest that diet and age have no effect on direction selectivity.

The mean CF for units in the A-BB group was 14.0 kHz (range 5.5–23.0 kHz), the A-CC group was 13.3 kHz (range: 5.5–25.5 kHz) and the A-CR group was 14.7 kHz (range: 3.0–25.0 kHz) respectively. We used the Bonferroni correction to compare CF between the different groups. This changed the level of significance to $p < 0.0083$ for the number of tests we performed. With this criterion, there were no significant differences in CF between Y-CR rats and aged rats in the A-BB ($p = 0.017$), A-CC ($p = 0.017$) or A-CR groups ($p = 0.053$). In addition, there were no significant differences in CF between the aged groups maintained on the three diets: A-BB versus A-CR ($p = 0.318$), A-BB versus A-CC ($p = 0.889$) and A-CC versus A-CR ($p = 0.318$).

3.3. Correlation of FM sweep parameters

Preferred speed of FM sweep appeared to be independent of CF for the two diet groups as well as for both the

young and aged (A-CR) rats (Y-CR: $r = 0.082$, $p = 0.565$; A-BB: $r = 0.007$, $p = 0.948$; A-CC: $r = -0.116$, $p = 0.292$; A-CR: $r = 0.148$, $p = 0.235$). In addition, DS appeared to be independent of CF for all groups of rats (Y-CR: $r = -0.257$, $p = 0.075$; A-BB: $r = 0.167$, $p = 0.124$; A-CC: $r = 0.052$, $p = 0.635$; A-CR: $r = -0.198$, $p = 0.112$).

Finally, there was no systematic relationship between a cell's preferred speed and direction selectivity as a function of diet or age (Y-CR: $r = -0.019$, $p = 0.893$; A-BB: $r = 0.142$, $p = 0.191$; A-CC: $r = 0.01$, $p = 0.991$; A-CR: $r = 0.009$, $p = 0.944$). This suggests that speed and direction are processed independently of each other as well as of diet and age.

4. Discussion

The results of the present study show that age-related changes in temporal processing speed in the auditory cortex of the rat may be reversed by an antioxidant-enriched diet. Specifically, cortical cells recorded from aged rats maintained on a diet supplemented with blueberry phytochemicals exhibited speed preferences for faster FM sweeps similar to those recorded from young rats.

4.1. Comparison with studies in young animals

The present study found that the majority of units recorded from aged rats fed the blueberry-enriched diet for 8 weeks preferred the faster FM sweep rates. Cells recorded from the IC, vMGN and A1 in young animals show a similar preference for FM sweeps [12,23,26,29,30,33,39]. What is intriguing about the present study is that the percentage of units preferring faster sweeps in the A-BB group was not only significantly greater than that observed in the A-CC and A-CR groups, but it was also greater than what we had previously found in young (Y-CR) rats. That is, instead of merely reversing or delaying the decline in temporal processing associated with aging, antioxidants appear to have enhanced the temporal processing capability relative to that observed in young rats. It is possible that this augmentation may be due to changes in neuronal signaling. Recently, we demonstrated that neuronal signaling in a transgenic mouse model of Alzheimer's disease could actually be enhanced by blueberry supplementation to the diet [15]. We have suggested that extracellular signal-regulated kinase (ERK) and protein kinase C (PKC) may be involved in this signal enhancement [15]. Thus, it is possible that in the present study, signaling in the experimental rats remained intact or was enhanced to the point where more neurons could process rapidly changing acoustic stimuli. Furthermore, it may be that the enhanced signaling facilitated neural synchrony thereby enhancing processing speed. In future, it would be both beneficial and interesting to compare these results with those obtained from young rats maintained on a blueberry-enriched.

In the present study, we found no significant correlation between CF and PS or DS. This is consistent with previous investigations [32,33,39]. However, it has recently been reported that a significant correlation between DS and CF in AI of young rats exists [60]. One possible explanation for this apparent difference may be the relatively small number of units recorded from each animal in the present study. These investigators did not report on a possible interaction between PS and CF [60].

4.2. Comparison with studies in aged animals

The two control groups (A-CC and A-CR) exhibited speed preferences for slow FM sweeps, similar to what we have observed in cortical units of aged rats [32]. However, as we have previously reported, this stands in contrast to what we have observed in other parts of the auditory pathway. In both the IC and MGN of aged rats, a relatively small percentage of the cells (IC: 24%; MGN:15%) prefer the slow speeds [23,26]. Collectively, these results support the hypothesis that the decline or delay in onset in temporal processing speed for acoustic stimuli, specifically FM sweep speed, may be a cortical phenomenon. This is further supported by other studies, which have shown that other forms of temporally modulated stimuli do not reveal age-related differences in units at the level of the IC [38]. Moreover, these cortical changes can be reversed by blueberry phytochemical dietary supplementation.

It should be mentioned that while we observed changes in the cortex, we cannot at present discriminate between changes that may be occurring subcortically. What we can say is that there was a change in temporal processing speed in the auditory cortex of aged results that had been maintained on a blueberry-supplemented diet. In addition, while we have not previously found age-related effects of temporal processing speed subcortically, it is possible that had we used a broader range of speeds, a difference may have been revealed. However, the likelihood of this is not very great since studies from other laboratories using other types of temporally modulated stimuli have found no age-related differences at least at the level of the IC [38].

4.3. Comparison with other antioxidant studies

While there have been relatively few reports on the effects of antioxidants in the auditory system studies have shown a deleterious effect of ROS on peripheral auditory structures [28,48]. In one study lecithin was given as a dietary supplement for 6 months to Harlan-Fischer rats aged 18–20 months [47]. The researchers found that auditory brainstem responses recorded from treated rats were significantly different from those of the control rats such that deterioration in hearing sensitivity was noted only in the control rats.

While the present study is the first to look at the effects of blueberry phytochemicals on sensory processing, the results are consistent with what has been reported for the effects

of these antioxidants on cognitive and motor behaviours. These studies have shown an improvement in cognitive and motor behaviours in aged rats that have been maintained on an antioxidant-enriched diet for 8 weeks [1,15,17]. Further, researchers have shown that an addition of only 2% of blueberry extract to the regular diet was sufficient to result in an improvement in these behaviours [58].

4.4. Neural mechanisms of oxidative stress

One mechanism of oxidative stress may involve changes in membrane potentials. For example, research suggests that age-related changes in neuronal plasma membrane molecular structure and increased rigidity may play a role in increasing vulnerability to OS [17,18]. In addition, one of the most detrimental effects of free radicals is impairment in membrane fluidity and elasticity due to lipid peroxidation [18,55]. That is, ROS can cause disruption in the plasma membrane resulting in leakage and thereby lead to a potential change in cell excitability. In the auditory cortex, we previously demonstrated that neural inhibition contributed to the preferred speed response of cells [30]. Specifically, we found that inhibition appeared to impose a preference for slower speeds. Thus, it is possible that in the auditory cortex of A-CC and A-CR rats, free radicals cause units to become hyperpolarized, which, in turn, leads to inhibition resulting in a preference for slower speeds. Antioxidants may in turn help to prevent this from occurring.

Oxidative damage to nucleic acids, predominantly RNA, has also been reported in the aged rat brain [25]. This form of damage could result in errors in translation thereby compromising protein synthesis and causing specialized proteins, such as ion channels, to become non-functional. Researchers have reported that the activity of Kv1.4, the K^+ channels found in neurons, is inhibited by ROS [6]. They hypothesized that modifications of K^+ channel activity by ROS may lead to detrimental changes in the electrical excitability of the neuron and affect the synchronous firing that occurs in neurons when they are stimulated in a given way. Studies have shown that neural synchrony between simultaneously discharging neurons may be one way in which the auditory system is able to distinguish between temporal cues [44]. Extrapolating this hypothesis to the present study, neurons must fire in a specific synchronous pattern in order for A1 cells to distinguish between the quick rates of frequency change. Hyperexcitable cells, however, may reduce the total number of simultaneously discharging neurons, thereby compromising neural firing patterns. This age-related disruption in neural synchrony may be partially responsible for the decline in temporal processing in the aged rat brain. It is possible that this disruption may be positively affected by blueberry supplementation.

Another mechanism that may play a role in OS is an increase in the number of activated microglial cells in the A1 of aged rats [53]. The process of phagocytosis is known to create ROS, which can then lead to a reduction of den-

dritic spines in aged cerebral cortex [36,52]. Therefore, a decrease in functional dendrites could lead to a reduction in the functional receptive area of a neuron. This may then limit the frequency range to which a neuron can respond because of a decrease in synaptic transmission efficacy. We previously proposed a link between modulation rate selectivity (i.e., FM sweep speed) and the width of a neuron's frequency response area [33]. We found that units with narrow frequency response areas had long integration times and therefore preferred slower modulation rates, while a preference for faster modulation rates was observed in neurons with broader frequency response areas. Thus, the aged AI cells of control rats in the present study may have a reduced response area resulting in longer integration of input and therefore a preference for slower FM speeds. Unfortunately, this is probably not the case since we recently found that there was no significant difference in the tuning curve properties (or Q_{10dB}) between auditory cortical cells recorded from young and old rats [32].

Another important role of an antioxidant may be its ability to interact with its intercompartmental components and to act synergistically with other antioxidants [7]. Consequently, in addition to performing their antioxidative duties, flavonoids could be working in concert with other phenolic compounds, natural antioxidants and endogenous enzymes in restoring homeostasis. Thus, once within the bloodstream, antioxidants may have the potential to act on oxidants within the body including those that affect the brain.

Recently, investigators found that anthocyanins contained in blueberries were located in those brain regions of aged rats associated with cognitive and motor performance, namely the hippocampus, striatum, cortex and cerebellum [1]. Although auditory cortical tissue was not specifically analyzed in that or the present study it is possible that the anthocyanins were acting in the cortical area.

Another possible mechanism involves changes in neuronal signaling as a result of flavonoids [27,43]. Phytochemicals, in particular, have been shown to regulate multiple signaling pathways at the level of transcription, especially those concerned with the mitogen activated protein kinase [9]. Recent studies have indicated that activation of the mitogen activated protein kinase cascade may be OS-sensitive [59]. These may serve as coincidence detectors for modulating coordinated responses to neuronal extracellular signals [51].

As mentioned above, ERK may have an age-related effect on cell signaling. We recently showed that BB supplemented middle-aged APP/PSI transgenic mice [5,13] showed greater levels of ERK and PKC in the hippocampus compared to control fed APP/PSI mice [15]. This suggests that BB supplementation may prevent cognitive deficits by enhancing neuronal signaling, particularly via mitogen activated protein kinases [15].

Finally, it is necessary to mention that at present, we do not fully understand how the anaesthetic used in the present study may affect the aging cortex or how it may interact with an antioxidant-enriched diet. For example, it is possible that Equithesan might have a cortically specific, detrimental effect

on sensitivity to high sweep rates in older rats in the control groups but not in the BB group. While this might seem unlikely, it cannot be ruled out.

5. Conclusion

In summary, it seems that free radical damage may be imparted on the cortex of aged rats. In particular, this type of age-related change in the cortex could have profound effects on neurotransmission and, consequently, on central nervous system processing of sensory information such as auditory stimuli. Such damage may have been reversed by the 2-month supplementation of dietary antioxidants in the form of blueberry phytochemicals. However, it is unclear if the same effects could have been attained had classical antioxidants, such as alpha-tocopherol or ascorbic acid been administered; to our knowledge such studies have not been performed. Furthermore, the exact mechanism by which flavonoids exert their antioxidant activity particularly in the auditory cortex is not clear and is the subject of future research.

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